

## Role of Selenium in Neural Physiology of Avian Species

### 2. THE DISTRIBUTION OF $^{75}\text{Se}$ FROM INJECTED SELENOMETHIONINE- $^{75}\text{Se}$ IN THE TISSUES AND ITS FATE IN LIVERS OF CHICKENS

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**ABSTRACT** Both male and female chickens were injected with a dose of selenomethionine- $^{75}\text{Se}$  two hours before killing and the total selenium and the radioactivity in various tissues were determined. For the sexes the tissues can be ranked as follows for total selenium content/gm. fresh tissue: (males) pituitary, pineal, adrenals, kidneys, liver, spleen, pancreas, retina, diencephalon, blood, cerebrum, testes, cerebellum and pectoral muscle; (females) pituitary, pineal, adrenals, kidneys, diencephalon, spleen, pancreas, liver, oviduct, cerebellum, ovary, cerebrum, pectoral muscle and blood.

The specific activity of  $^{75}\text{Se}$  was highest in testes, pancreas, liver, kidneys and spleen of males; and pancreas, oviduct, blood, kidneys and liver of females. About 82% of the radioactivity was associated with a T.C.A. insoluble fraction in liver.

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**D**URING their studies on the role of selenium in neural physiology of chickens, turkeys and coturnix, McFarland *et al.* (1970) determined its distribu-

tion from an injected dose of  $\text{H}_2^{75}\text{SeO}_3$  into various tissues. This selenium in tissues might have been in an ionic form as  $\text{H}_2\text{SeO}_3$  or undergone reduction to form compounds such as selenomethionine and its metabolites. If the reduction to selenomethionine was important, the distribution of  $^{75}\text{Se}$  may be different from an injected dose of selenomethionine- $^{75}\text{Se}$  than from  $\text{H}_2^{75}\text{SeO}_3$ . This possibility has been investigated in the present study.

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## EXPERIMENTAL METHODS

White Leghorn chickens raised on a commercial breeder diet and about 1.5 years of age were used in this study. The selenium content of this diet was found in the earlier study (McFarland *et al.*, 1970) to be 3.42 nanogram-atom per gram (0.27 p.p.m.). Each of the five males and five females was given an injection of a commercially available solution (1.5 ml.) of L-selenomethionine-<sup>75</sup>Se equivalent to 150 $\mu$ Ci. in the wing vein two hours before the bird was killed. L-Selenomethionine-<sup>75</sup>Se solution (specific activity 0.25 mCi./0.2 mg. selenomethionine/ml.) was obtained from Amersham/Searle Corp. Arlington Heights, Illinois. Each injection gave the bird 0.03 mg. selenomethionine or 156.25 nanogram-atom Se. The specific activity was equal to  $2.13 \times 10^6$  disintegration per minute (D.P.M.) per nanogram-atom of Se, which contributed insignificantly towards the endogenous Se of the bird.

The blood samples and tissues were transferred to tared vials for later analysis and the weight of the fresh tissue was determined. A deep-well gamma counter

(Tracer Lab., Inc.) was used for the measurement of radioactivity of the tissues. The method of Watkinson (1966) involving wet digestion of samples with HNO<sub>3</sub>-HClO<sub>4</sub> to determine total Se fluorometrically was used. For total Se, samples of pituitary and pineal were pooled.

Part of the livers from males were homogenized and an aliquot of the sample was separated into trichloroacetic acid (T.C.A.) soluble and insoluble fractions. The efficiency of counting was 29.3% and the counts per minute were converted to disintegrations per minute and the necessary corrections were also made for any decay of the isotope by counting a known volume of the original solution along with the samples.

The average values of the radioactivity along with the standard errors for various tissues are given in the tables.

## RESULTS AND DISCUSSION

*Endogenous Se Concentration:* The concentration of total selenium (nanogram-atom/gm. fresh tissue) is highly variable from tissue to tissue but in general the

TABLE 1.—The occurrence of total Se and incorporation from selenomethionine-<sup>75</sup>Se in fresh tissues of male White Leghorn chickens (average  $\pm$  standard error). (Efficiency of counting = 29.3%)

Tissue	Total Selenium nanogram-atom/gm.	Specific Activity, D.P.M. $\times 10^{-6}$		Rank according to Sp. Activity
		per gm. fresh tissue	ngm.-atom Se	
Pituitary	47.95 <sup>1</sup>	3.79 $\pm$ 0.20	0.08 $\pm$ .01	7
Pineal	23.98 <sup>1</sup>	2.94 $\pm$ 0.43	0.12 $\pm$ .02	6
Adrenals	12.94 $\pm$ 1.46	3.15 $\pm$ 0.30	0.25 $\pm$ .02	5
Kidneys	13.29 $\pm$ 1.24	4.14 $\pm$ 0.22	0.31 $\pm$ .02	4
Liver	9.25 $\pm$ 0.33	3.66 $\pm$ 0.15	0.40 $\pm$ .02	3
Spleen	9.20 $\pm$ 0.77	2.53 $\pm$ 0.18	0.29 $\pm$ .04	4
Pancreas	7.46 $\pm$ 0.99	8.27 $\pm$ 1.22	1.13 $\pm$ .12	2
Retina	4.94 $\pm$ 1.24	0.48 $\pm$ 0.05	0.12 $\pm$ .04	6
Diencephalon	4.88 $\pm$ 0.31	0.57 $\pm$ 0.03	0.12 $\pm$ .01	6
Blood	4.77 $\pm$ 0.29	1.00 $\pm$ 0.05	0.22 $\pm$ .02	5
Cerebrum	4.12 $\pm$ 0.39	0.52 $\pm$ 0.04	0.13 $\pm$ .01	6
Testes	3.94 $\pm$ 0.41	6.53 $\pm$ 0.66	1.67 $\pm$ .09	1
Cerebellum	3.57 $\pm$ 0.24	0.72 $\pm$ 0.06	0.20 $\pm$ .02	5
Pectoral Muscle	3.12 $\pm$ 0.18	0.27 $\pm$ 0.03	0.09 $\pm$ .01	7

<sup>1</sup> Pooled samples.

TABLE 2.—The occurrence of total Se and incorporation from selenomethionine-<sup>75</sup>Se in fresh tissues of female White Leghorn chickens (average  $\pm$  standard error) (Efficiency of counting = 29.3%)

Tissue	Total Selenium nanogram-atom/gm.	Specific Activity, D.P.M. $\times 10^{-6}$		Rank according to Sp. Activity
		per gm. fresh tissue	ngm.-atom Se	
Pituitary	29.0 <sup>1</sup>	4.65 $\pm$ 0.33	0.16 $\pm$ .01	6
Pineal	30.29 <sup>1</sup>	4.28 $\pm$ 0.40	0.14 $\pm$ .01	6
Adrenals	20.25 $\pm$ 5.50	2.62 $\pm$ 0.20	0.16 $\pm$ .04	6
Kidneys	12.32 $\pm$ 1.50	4.70 $\pm$ 0.43	0.41 $\pm$ .06	3
Diencephalon	11.27 $\pm$ 1.77	0.60 $\pm$ 0.02	0.06 $\pm$ .01	7
Spleen	11.41 $\pm$ 1.14	2.70 $\pm$ 0.14	0.24 $\pm$ .02	5
Pancreas	10.81 $\pm$ 0.71	10.18 $\pm$ 1.14	0.93 $\pm$ .07	1
Liver	10.29 $\pm$ 0.35	3.55 $\pm$ 0.30	0.34 $\pm$ .03	4
Oviduct	9.78 $\pm$ 1.45	5.40 $\pm$ 0.94	0.61 $\pm$ .16	2
Retina	8.50 $\pm$ 1.70	0.52 $\pm$ 0.04	0.07 $\pm$ .01	7
Cerebellum	7.64 $\pm$ 0.40	0.66 $\pm$ 0.04	0.09 $\pm$ .01	7
Ovary	6.27 $\pm$ 0.67	1.20 $\pm$ 0.06	0.20 $\pm$ .02	5
Cerebrum	6.10 $\pm$ 0.97	0.53 $\pm$ 0.01	0.11 $\pm$ .02	7
Pectoral muscle	4.40 $\pm$ 0.33	0.40 $\pm$ 0.05	0.09 $\pm$ .01	7
Blood	3.94 $\pm$ 0.36	1.52 $\pm$ 0.11	0.40 $\pm$ .01	3

<sup>1</sup> Pooled samples.

maximum concentrations were in pituitary and pineal. The various tissues are ranked according to the concentrations of total selenium from the highest to the lowest for male and female chickens in Table 1 and 2. The selenium concentration is highly variable in males and females even in those tissues which have its highest concentration. In contrast to the earlier observations with  $H_2^{75}SeO_3$  (McFarland *et al.*, 1970) the female chickens had a higher total selenium in pineal than males. Pituitary, pineal, adrenals and kidney were again found to be the most concentrated sources of selenium.

The concentration of selenium in blood was more than in cerebrum and cerebellum in males but less in females in this experiment. It is doubtful that this has any meaning because of the short duration of the experiment.

The specific activity expressed in terms of D.P.M.  $\times 10^{-6}$  per nanogram-atom present in the various tissues are also given in Tables 1 and 2. The average body weights of male and female chickens were  $2030 \pm [123 \text{ (S.E.)}]$  and  $1627 \pm [125$

(S.E.)] gm., respectively but the injected dosage of selenomethionine-<sup>75</sup>Se was the same in all cases. This accounts for a higher specific activity of blood selenium of female chickens. The rank of tissues in the order of decreasing specific activities is not the same as the rank according to the concentration of total selenium. Again, the tissues of male and female chickens did not have the parallel ranks as regards the concentration of selenium-<sup>75</sup> from selenomethionine.

The testes, pancreas followed by liver, kidney and spleen had the highest specific activity in males. In females, pancreas, oviduct, were followed by kidney, blood, and liver, as regards the specific activity.

Generally speaking, the important sites

TABLE 3.—The distribution of radioactivity from selenomethionine-<sup>75</sup>Se in the T.C.A.-soluble and insoluble fractions of chicken liver (average  $\pm$  standard error)

Fresh Liver from Males	
D.P.M./gm.	187 $\pm$ 14
T.C.A.-soluble fraction, D.P.M.	37 $\pm$ 8
T.C.A.-insoluble, D.P.M.	166 $\pm$ 13
Recovery, %	109 $\pm$ 8
Protein-bound, <sup>75</sup> Se, %	83 $\pm$ 3

of localization of selenium-<sup>75</sup> from selenomethionine are testes, pancreas, oviduct, liver and kidney.

It is suggested that selenium is concentrated in organs such as the pancreas, testes, oviduct, liver, kidneys. In the gonads, the synthesis of proteins associated with sperm and egg is taking place and this may explain the high levels of <sup>75</sup>Se from selenomethionine. The other organs are involved in metabolism and elimination of selenomethionine.

Most of the selenium-<sup>75</sup> activity was in form of protein-bound fraction (83%) in the liver (Table 3).

#### REFERENCES

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